REMARKS

Claims 1-5 are pending in the application, and stand rejected by the Examiner. For the reasons set forth below, Applicants respectfully traverse.

Specification

Applicants acknowledge the Examiner's removal of all objections to the specification.

Rejection Under 35 U.S.C. §101

The Examiner has maintained the rejection of pending Claims 1-5 under 35 U.S.C. § 101 as lacking utility for the reasons set forth in the previous Office Actions. The Examiner states that the specification discloses that the PRO300 polynucleotide is more highly expressed in normal lung tissue as compared to lung tumor, and that Applicants have asserted the use of the polypeptide for diagnosis. However, the Examiner rejects this utility, stating that "one cannot [on the basis of utility of an encoding nucleic acid] support a utility for the encoded protein and antibody which binds it because the prior art provides sufficient support to make a correlation between mRNA and encoded protein level unpredictable." *Final Office Action* at 3.

Applicants incorporate by reference their previously submitted arguments, and for the reasons of record assert that the specification contains a disclosure of utility and therefore must be taken as sufficient to satisfy the utility requirement of 35 U.S.C. § 101. Applicants also submit that for reasons of record, the Examiner has not met the PTO's burden of providing evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility. However, even if the Examiner has met the PTO's initial burden, Applicants' rebuttal evidence previously submitted and additional evidence submitted herewith is sufficient to prove that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated previously, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

Substantial Utility

Summary of Applicants' Arguments and the Examiner's Response

Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO300 polypeptide is expressed at least two-fold higher in normal lung tissue as compared to lung tumor tissue;

2. Applicants assert that it is well-established in the art that a change in the level of mRNA for a particular protein, *e.g.* a decrease, generally leads to a corresponding change in the level of the encoded protein, *e.g.* a decrease; and

3. Given the differential expression of the PRO300 mRNA in lung tumors compared to normal lung tissue, it is more likely than not that the PRO300 polypeptide is also differentially expressed in lung tumors compared to normal lung tissue, making the claimed antibodies useful as diagnostic tools, alone or in combination with other diagnostic tools.

Applicants understand the Examiner to be making three arguments in response to Applicants' asserted utility:

1. The Examiner challenges the reliability of the evidence reported in Example 18, stating that the specification does not provide "critical information" relating to the assay in Example 18;

2. The Examiner argues that Hu *et al.* (J. Proteome Res., (2003) 2:405-412), and Saito-Hisaminato *et al.* (DNA Res. (2002) 9:35) demonstrate that the literature cautions researchers from drawing conclusions based on small changes in transcript expression levels; and

3. The Examiner cites Hu *et al.* and Haynes *et al.* (Electrophoresis, (1998) 19(11):1862-71), Chen *et al.* (Mol. and Cell. Proteomics, (2002) 1:304-313) and Fessler *et al.* (J. Biol. Chem., (2002) 277(35): 31291-31302); to support her position that polypeptide levels cannot be accurately predicted from mRNA levels. According to the Examiner, further research needs to be done to determine if the increase or decrease in PRO300 cDNA expression supports a role for the peptide in cancerous tissue.

As set forth below, in light of all of the evidence, the Examiner's arguments are not adequate to support the utility rejection of the claimed invention under 35 U.S.C. § 101.

Example 18 and the Grimaldi Declaration Establish that Differential Expression of mRNA is Sufficient to Provide Utility for the mRNA as a Diagnostic Tool

Applicants first address the Examiner's argument that the evidence of differential expression of the gene encoding the PRO300 polypeptide in lung tumors is insufficient, and that the literature cautions against drawing conclusions based on small changes in transcript expression levels between normal and cancerous tissue.

The Examiner states that the data in Example 18 is insufficient to support a substantial utility for the claimed antibodies since "interpretation of the [differential expression] results depends, for example, on relative or absolute levels of the difference(s), the ability to generalize to more than one cell culture or tumor type or, conversely, the ability to pinpoint a particular tumor type (*e.g.* adenocarcinoma *versus* squamal), and the repeatability of the differential expression both in terms of frequency/prevalence and quantity/sensitivity." *Office Action* at 5. The Examiner argues that "different types of lung tumors exhibit different patterns of gene expression [and] [l]ung tumors in different stages of cancer can exhibit different patterns of gene expression as well as various types of lung cancer". *Id.* The Examiner fails, however, to proffer evidence or reasoning why the alleged lack of information regarding lung tumor *type* or *stage* renders the data in Example 18 insufficient to support Applicants' claimed utility. *Id.* at 11. These unsupported objections cannot establish a *prima facie* case. *See In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995) ("Only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant to provide rebuttal evidence.") (emphasis added). Applicants have not asserted that the claimed antibodies are useful as diagnostic tools for adenocarcinoma *versus* squamal lung tumors. Similarly, Applicants have not asserted that the claimed antibodies are useful as diagnostic tools for a specific stage of lung tumor. Rather, Applicants have asserted that the claimed antibodies are useful as diagnostic screening tools for the detection of lung tumor. As discussed in the first Grimaldi Declaration (submitted as Exhibit 1 in the Amendment and Response to Office Action mailed January 3, 2005), the data in Example 18 were generated from cDNA libraries from pooled samples of normal and of tumor tissues. Thus, the data in Example 18 "indicate that the genes of interest [*e.g.*, the gene encoding PRO300] can be used to differentiate tumor from normal." *First Grimaldi Declaration* at ¶ 7. (Emphasis added) Mr.

Grimaldi testifies that "the precise type of tumor is [] irrelevant. . .the assay was designed to indicate whether a difference exists between normal tissue and tumor tissue of the same type." *First Grimaldi Decl.* at ¶ 7. Because Applicants are not asserting that the claimed antibodies are useful diagnostic tools for, *e.g.*, adenocarcinoma or a particular stage lung tumor, the Examiner's requirement that Applicants' provide information regarding the specific type of and stage of lung tumors tested in Example 18 is improper. The fact that Applicants' provide data from pooled samples of lung and normal tissue, and do not differentiate type or stage of tumor is irrelevant to and does not detract from Applicants' asserted utility.

The only objection by the Examiner to the data in Example 18 that is supported by any reasoning or evidence is the assertion based on the Hu *et al.* and Saito-Hisaminato *et al.* references that the literature cautions researchers from drawing conclusions based on small changes in transcript expression levels between normal and cancerous tissue. As set forth below, these references do not cast doubt on the data in Example 18 or Applicants' asserted utility. Applicants have previously pointed to the teachings of the specification and the first Grimaldi Declaration establishing that the teachings of the specification are sufficient to establish that the PRO300 gene is under-expressed in tumor cells compared to corresponding normal tissue. Applicants incorporate by reference the previous arguments, and will not repeat them here. The Examiner has rejected Mr. Grimaldi's testimony, maintaining that the Grimaldi Declaration does not teach the level of reliability of the results in Example 18, that no relative or absolute levels of PRO300 mRNA in control or tumor tissue are disclosed, that Hu *et al.* and Saito-Hisaminato *et al.* provide strong evidence that the literature cautions researchers from drawing conclusions based on small changes in transcript expression levels, and that Mr. Grimaldi is an inventor on the application.

Applicants first address the Examiner's argument that the Grimaldi Declaration is insufficient because it does not teach the reliability, or relative or absolute levels of PRO300 mRNA in control or tumor tissue. As indicated in the first Grimaldi Declaration, differential gene expression studies concern "genes whose expression levels differ *significantly* under different conditions, for example in normal versus diseased tissue." *First Grimaldi Decl.* at ¶ 6. Mr. Grimaldi also testifies that "[t]he precise levels of gene expression are irrelevant; what matters is that there is a *relative* difference in expression between normal tissue and tumor

tissue." *Id.* at ¶ 7. In other words, the expression data in Example 18 is a reliable indicator of differential expression of genes (*e.g.*, the gene encoding PRO300) in lung tumor tissue compared to normal lung tissue. As discussed below, the evidence offered by the Examiner, *i.e.*, Hu *et al.* and Saito-Hisaminato *et al.*, does not lead the skilled artisan to question Applicants' asserted utility, or the testimony of Mr. Grimaldi.

Applicants have previously discussed why Hu, which is directed to a known role in a disease, is not relevant to the issue of whether the differentially expressed PRO300 mRNA is useful as a diagnostic tool for cancer. Applicants incorporate by reference the previous arguments, and will not repeat them here.

In addition to the persuasive reasons articulated in Applicants' arguments of record, the Examiner's reliance on Hu is also misplaced because Applicants are not relying on microarray data as discussed in Hu:

> In any microarray experiment, thousands of genes may demonstrate statistically significant expression changes, but only a fraction of these may be relevant to the study. *Hu* at 405, left column, first paragraph (emphasis added).

Instead, Applicants are relying on quantitative PCR analysis to measure gene expression levels. In a recent study by Kuo *et al.* (Proteonmics 5(4):894-906 (2005)), the authors used microarray analysis combined with proteomic analysis using two-dimensional gel electrophoresis to examine changes in gene expression in leukemia cell lines. The authors report that "[c]omparison of microarray and proteomic expression profiles showed poor correlation. Use of more reliable and sensitive analyses, such as reverse transcriptase polymerase chain reaction [RT-PCR], Western blotting and functional assays, on several genes and proteins, nonetheless, confirmed that there is indeed a good correlation between mRNA and protein expression." *Kuo et al.* at Abstract (emphasis added)(attached as Exhibit 1). Thus, even if accurate, Hu's statements regarding microarray studies are not relevant to the instant application which does not rely on microarray data.

Next, the Examiner argues that Saito-Hisaminato *et al.* demonstrates that the data in Example 18 are insufficient to support Applicants' asserted utility. According to the Examiner Saito-Hisaminato *et al.* "demonstrate that among 23,040 genes studied in normal human tissue, 4080 genes were highly expressed (greater than 5-fold higher than in other tissues) in one, or only a few tissues (see abstract, lines 3-5). This represents about 18% of the total genes tested.

Saito-Hisaminato *et al.* only disclose genes that are highly expressed in one tissue compared to other tissues (*i.e.*, greater than 5-fold difference). A 5-fold difference is greater than the difference required to establish that a gene is 'overexpressed' or 'differentially expressed' by comparison in the specification." *Office Action* at 13-14.

Applicants fail to see the relevance of Saito-Hisaminato *et al.* to Applicants' asserted utility. Saito-Hisaminato *et al.* reported genes that showed a 5-fold difference using cDNA microarrays from different tissues. Saito-Hisaminato *et al.* is completely silent regarding the "reliability" or "significance" of differential expression that is less than 5-fold. The fact that the authors in Saito-Hisaminato *et al.* chose a particular numerical cutoff for purposes of reporting their results does not mean that different numerical cutoffs are insignificant or unreliable. Further, Saito-Hisaminato *et al.* is completely silent regarding the reliability of differential expression data generated using quantitative PCR, the technique used in Example 18. Because Saito-Hisaminato *et al.* does not address the significance or reliability of quantitative PCR results, it does not "constitute strong opposing evidence" to the Grimaldi Declaration. Rather, Saito-Hisaminato *et al.* carries no evidentiary weight.

In conclusion, Applicants submit that the evidence reported in Example 18, supported by the first Grimaldi Declaration, establish that there is at least a two-fold difference in PRO300 mRNA between lung tumors compared to normal lung tissue. The teachings of Hu are not relevant to Applicants' asserted utility because Hu is directed to the sufficiency of microarray data in identifying genes with a known role in a disease, and Applicants have asserted that the claimed antibodies have a diagnostic utility, based in part on the results of the PCR analysis of Example 18. Similarly, the teachings of Saito-Hisaminato *et al.* are not relevant to Applicants' asserted utility because they do not address the reliability of quantitative PCR differential gene expression data. Therefore, the only issue which remains is whether the data in Example 18 regarding differential expression of the PRO300 mRNA are reasonably correlated with differential expression of the PRO300 polypeptide such that the claimed antibodies have utility as diagnostic tools as well. As discussed below, even if the Examiner has established a reasonable doubt regarding Applicants' assertion that they are reasonably correlated, Applicants' overwhelming rebuttal evidence is more than sufficient to establish that changes in mRNA level lead to corresponding changes in protein level.

The Examiner's Evidence is Not Relevant to Determining Whether a Change in mRNA Level for a Particular Gene leads to Corresponding Changes in the Level of the Encoded Protein

Applicants turn next to the second portion of their argument in support of their asserted utility – that it is well-established in the art that a change in the level of mRNA encoding a particular protein generally leads to a corresponding change in the level of the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO300 polypeptide in lung tumors, it is likely that the PRO300 polypeptide is also differentially expressed; and proteins differentially expressed in certain tumors have utility as diagnostic tools.

In response to Applicants' assertion, the Examiner cites Haynes *et al.* (Electrophoresis, (1998) 19(11):1862-71), Chen *et al.* (Mol. and Cell. Proteomics, (2002) 1:304-313) and Fessler *et al.* (J. Biol. Chem., (2002) 277(35):31291-31309) as support for the argument that "one of ordinary skill in the art would not reasonably assume [that expression of a protein will correlate with mRNA expression] in the absence of evidence." *Office Action* at 17.-

As to the Examiner's cited references, Applicants have previously discussed at length why the Haynes and Chen and references are not relevant to the issue of whether changes in mRNA level for a particular gene lead to changes in protein level. Applicants incorporate by reference the previous arguments, including those made in their appeal brief, and will not repeat them here.

However, in an attempt to illustrate why references which relate to static global levels of mRNA and protein across different genes are not relevant to this issue, Applicants offer the following illustration and analogy with the understanding that like all illustrations and analogies, they are not perfect and therefore do not represent any admissions or binding statements regarding Applicants' disclosure or invention.

Haynes *et al.* as well as portions of Chen *et al.* discuss whether there is a correlation between the static level of mRNAs and proteins globally, *i.e.* across different genes. This is equivalent to conducting a hypothetical Experiment 1, where a particular cell type has 100 copies of mRNA for gene X, 200 copies of mRNA for gene Y, and 400 copies of mRNA for gene Z. If there is a global correlation between static mRNA levels and protein levels across genes, the ratio

of the amount of proteins X:Y:Z would be approximately 1:2:4. This is essentially what the cited references examined.

In contrast, Applicants are relying on a correlation between changes in mRNA level for a particular gene leading to a corresponding change in the level of the encoded protein. For example, in hypothetical Experiment 2, if gene X has 200 copies of mRNA per cell in condition A (*e.g.* normal), and 100 copies of mRNA for gene X in condition B (*e.g.* tumor), the ratio of the amount of protein X in condition A:B would be approximately 2:1, such that there is a correlation between the change in the level of mRNA and protein for a particular gene.

The Examiner argues that because there is no correlation between static levels of mRNA and protein across genes, as illustrated by Experiment 1, one of skill in the art would not expect an increase or decrease in the amount of mRNA for a particular gene to result in a corresponding change in the amount of the encoded protein, as illustrated in Experiment 2. This is simply wrong.

For example, Haynes *et al.* reports that the amount of protein produced by similar levels of mRNA varied by as much as fifty-fold, and that similar amounts of protein were sustained by amounts of mRNA that varied by as much as forty-fold. *Haynes et al.* at 1863, first full paragraph. Based on these results, Haynes concludes that "protein levels cannot be accurately predicted from the level of the corresponding mRNA transcript." *Id.*

This is analogous to a finding that on one gallon of gas, a hybrid car can travel 70 miles but a large truck can only travel 5 miles, or that to travel 70 miles, a hybrid car requires 1 gallon of gas, but a large truck requires 14 gallons. That is to say, there are many things which affect the fuel efficiency of an automobile. Based on these observations, one could conclude that given the lack of correlation between the amount of gas in an automobile and the distance it travels, one cannot predict how far an automobile will travel based on the amount of gas in the tank.

Even if true, the Haynes *et al.* data and conclusions are irrelevant to Applicants' assertion, which is that increasing or decreasing the amount of mRNA for a particular gene will result in a corresponding increase or decrease in the amount of the encoded protein. This is analogous to increasing or decreasing the amount of gas in an automobile – it will travel farther if you add more gas, and not as far with less. The fact that there are many things which affect fuel efficiency and therefore you cannot predict how far and automobile will travel without knowing

if it is a hybrid or a large truck is irrelevant – both a hybrid and a truck travel farther on more gas, and not as far on less.

Similarly, Chen *et al.* report that plotting the level of mRNA for a particular gene against the level of the corresponding protein as measured across numerous samples, they found a lack of correlation for most genes studied. *Chen et al.* at Abstract. However, with the exception of three genes reported in Figures 2A-2C, Chen *et al.* does not indicate whether the level of mRNA varied significantly across samples, and Chen did not select samples or genes which were expected to vary across samples (*e.g.* normal versus tumor). Therefore, it is not known if Chen *et al.* examined changes in mRNA level, or if the level of mRNA was unchanged. Therefore, the relevance of Chen's finding to Applicants' asserted correlation between changes in mRNA and protein is not known.

By analogy, if a person drives a particular car as far as possible on 5 gallons of gas 20 different times, and then plots the amount of gas against the distance driven, a lack of correlation between the amount of gas and distance is meaningless, and merely reflects systematic error in measuring the amount of gas and distance driven. Only if substantially different amounts of gas were plotted against their respective distances can you answer the question of whether increasing or decreasing the amount of gas results in increasing or decreasing the distance driven.

Applicants emphasize, and the Examiner will recognize, that these are simplified illustrations to demonstrate the difference between the two issues being examined. However, these illustrations make clear that even if there is no correlation in the first experiment looking at static levels of mRNA and protein across genes, there can still be a correlation between changes in mRNA and protein for a particular gene as examined in the second experiment. As these illustrations make clear, the Examiner's evidence simply is not relevant to answering the question of whether it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true.

A change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein. Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' assertion. Nevertheless,

the Examiner focuses on the data in Fessler *et al.* in which mRNA levels are unchanged as well as data where mRNA levels were reported "absent".

As previously discussed, Fessler *et al.* studied changes in neutrophil (PMN) gene transcription and protein expression following lipoplysaccharide (LPS) exposure. Fessler *et al.* lists in Table VIII a comparison of the change in the level of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of 13 up-regulated proteins, Fessler *et al.* report a change in mRNA levels in only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Thus, in 2 out of 3 cases, an increase in mRNA correlated with an increase in protein levels. Of 5 down-regulated proteins, a change in mRNA is reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in every case, a decrease in mRNA correlated with a decrease in protein levels. The data in which a change in mRNA levels is reported are the only data that are relevant to Applicants' asserted utilities. In 5 of the 6 cases for which a change in mRNA levels are reported, the change in the level of mRNA corresponds to the change in the level of the protein. This is consistent with Applicants assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

The remainder of the proteins listed in Table VIII relate to instances where protein levels changed while mRNA levels were unchanged. The Examiner asserts that "what the results of Fessler *et al.* show is that a change in mRNA level does not necessarily have a corresponding change in protein levels and *vice versa.*" *Office Action* at 25. Applicants submit that Fessler *et al.* demonstrate that in 5/6 cases (*i.e.* 83% of the time), a change in mRNA correlates with a change in protein levels. Applicants make no assertion as to whether or not changes in protein levels "necessarily" correlate with changes in mRNA levels. This evidence has no relevance to Applicants' assertion that changes in mRNA levels lead to corresponding changes in protein levels, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. In accounting for these results, Fessler *et al.* explains that LPS has post-transcriptional activity that can influence protein levels (Fessler *et al.* at 31300, right column). Nothing in these results by Fessler *et al.* suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results are not contrary to Applicants' assertions.

In the final 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertion that changes in mRNA levels causes corresponding changes in protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased or decreased in PMN upon contact with LPS. Regarding these instances, Fessler *et al.* explains that LPS may have post-translational activity that can result in increased protein stability (Fessler at 31300, right column). Nothing in these results by Fessler *et al.* suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results also are not contrary to Applicants' assertions.

Thus, Fessler's results suggest that LPS has a transcriptional activity that can cause changes in mRNA levels which correlate with changes in protein levels, and that LPS also has post-transcriptional activity that can cause changes in protein levels that are not related to changes in mRNA levels. Accordingly, Fessler's results are consistent with Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Even if Fessler's results had shown that a change in the level of mRNA did not generally lead to a corresponding change in the level of the encoded protein, which they did not, the accuracy of Fessler's results is uncertain. Fessler *et al.* states that there were "limitations" to the results reported. These limitations included: possible artifactual transcript-protein discordance due to a 4 hour delay in harvesting after LPS exposure; uncertain post-incubation but pre-electrophoresis effects on protein synthesis, degranulation and exocytosis; and limited ability to quantitate protein amounts using Coomassie Blue. (Fessler *et al.* at 31301, left column). Fessler *et al.* exemplifies one such spurious result, in which there was a disparity between observed increase in cytokine mRNA, but an absence of detected cytokine proteins, which, as Fessler *et al.* explains, "reflects their removal in the post-LPS incubation wash." (Fessler *et al.* at 31297, right column). Thus, Fessler *et al.* acknowledges "limitations" to the conclusion that, for some genes, transcript levels did not coincide well with corresponding protein levels, leaving it uncertain the extent to which actual changes in protein levels differed from mRNA levels when neutrophils were exposed to LPS.

As such, Fessler *et al.* does not represent "influential art ... that requires the Examiner maintain that as a whole, the prior art does not provide a reasonable expectation that expression of the nucleic acid of SEQ ID NO:11 positively correlates with the expression of the protein of SEQ ID NO:12." Office Action at 9. Instead, Fessler *et al.* represents a teaching that LPS might cause transcriptional changes that correlate with changes in protein levels, and might also cause post-transcriptional changes in protein levels when mRNA levels are unchanged. Accordingly, Fessler *et al.* is not contrary to Applicants' asserted utility.

Applicants' Evidence Establishes that a Change in mRNA Level for a Particular Gene lead to Corresponding Change in the Level of the Encoded Protein

In support of the assertion that changes in mRNA are positively correlated to changes in protein levels, Applicants previously submitted a copy of a second Declaration by J. Christopher Grimaldi, a copy of the declaration of Paul Polakis, Ph.D., excerpts from the Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) and (4th ed. 2002), excerpts from the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)), a reference by Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004, and a reference by Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002). The details of the teachings of these declarations and references, and how they support Applicants' asserted utility, are of record and will not be repeated here.

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a comprehensive study by Ornoft *et al.* (Mol. Cell. Proteomics. 2002; 1(1):37-45) (previously submitted with IDS, attached hereto as Exhibit 2), the authors examined gene amplification, mRNA expression level, and protein expression in pairs of non-invasive and invasive human bladder tumors. *Id.* at Abstract. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations. Only one gene showed disagreement between transcript alteration and protein alteration." *Id.* at 42, col. 2. The alternations in mRNA and protein included both increases and decreases. *Id.* at 43, Table II. Clearly, a correlation in 39 of

40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (abstract attached as Exhibit 3) the authors report that down-regulation of E-cadherin protein has been shown in various human tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and nonneoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (abstract attached as Exhibit 4) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 5) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1

mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 6) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (abstract attached as Exhibit 7) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 8). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Mizrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 9) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 10), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-

regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, e.g. a decrease, lead to a corresponding change in the level of the encoded protein, e.g. a decrease.

In an article by Guo and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 11) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, e.g. an increase, generally leads to a corresponding change in the level of protein expression, e.g. an increase.

These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an additional 70 references (abstracts attached as Exhibit 12) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer indirect support of Applicants' asserted utility. As discussed in detail above, Applicants have challenged the relevance of references such as Haynes *et al.* and Chen *et al.* which do not attempt to examine the correlation between a change in mRNA level and a change in the level of the corresponding protein level. Because the Examiner continues to rely on these references, Applicants are submitting references which report results that are contrary to the Examiner's cited references and offer indirect support for Applicants' asserted utility.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (abstract attached as Exhibit 13) the authors conducted a study of mRNA and protein expression in yeast which was nearly identical to the one conducted by Gygi *et al.* and reported in Haynes *et al.* Contrary to the results of the earlier study by Gygi, Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box

gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 15) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 16) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 17) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 18) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the Examiner's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 20) which also support Applicants' assertion in that the references report a correlation between the level of mRNA and corresponding protein, contrary to the Examiner's assertion that mRNA and protein levels are not correlated.

In summary, Applicants submit herewith a total of 113 references in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein.

Applicants also previously submitted the Polakis Declaration in support of their position that in general, changes in mRNA levels correlate with changes in protein levels. (Submitted as Exhibit 3 in the Amendment and Response mailed January 5, 2005). In response, the Examiner merely states that "there is sound supporting evidence showing the unpredictability of saying level of expression of a particular nucleic acid will correlate with expression of the encoded protein." *Office Action* at 15. In an effort to expedite prosecution in this case, Applicants submit herewith as Exhibit 21 a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew. *In re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985). "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to

persuasiveness of argument" *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir. 1996)(quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)). Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner" *Id.* at 1583. Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." Part IIB, 66 Fed. Reg. 1098 (2001).

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 22). However, Applicants remind the Examiner that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that because the PRO300 mRNA is differentially expressed in lung tumors as compared to normal lung tissue, the PRO300 polypeptide will likewise be differentially expressed in lung tumors. This differential expression of the PRO300 polypeptide makes the claimed antibodies useful as diagnostic tools for cancer, particularly lung cancer.

Specific Utility

The Examiner maintains that "while it is agreed that the asserted utility for the polynucleotide of SEQ ID NO: 11 would be considered specific. . .it is not agreed that the polypeptide of SEQ ID NO: 12 does [*sic*]." *Office Action* at 31. According to the Examiner, Applicants' asserted utility is not specific due to the alleged lack of information in the disclosure. Applicants respectfully disagree.

Specific Utility is defined as utility which is "specific to the subject matter claimed," in contrast to "a general utility that would be applicable to the broad class of the invention." *M.P.E.P.* § 2107.01 I. Appellants submit that the evidence of differential expression of the PRO300 gene and polypeptide in lung tumor cells, along with the declarations and references discussed above, provide a specific utility for the claimed antibodies.

As discussed above, there are significant data which show that the gene for the PRO300 polypeptide is expressed at least two-fold higher in normal lung tissue compared to lung tumor. These data are strong evidence that the PRO300 gene and polypeptide are associated with lung tumors. Thus, contrary to the assertions of the Examiner, Appellants have provided evidence associating the PRO300 gene and polypeptide with a specific disease. The asserted utility for polypeptides related to the PRO300 polypeptide as diagnostic tools for cancer, particularly lung tumor, is a specific utility – it is not a general utility that would apply to the broad class of antibodies.

Utility – Conclusion

Applicants remind the Examiner that the evidence supporting utility does not need to be direct evidence, nor does it need to provide an exact correlation between the submitted evidence and the asserted utility. Instead, evidence which is "reasonably" correlated with the asserted utility is sufficient. *See Fujikawa v. Wattanasin*, 93 F.3d 1559, 1565, 39 U.S.P.Q. 2d 1895 (Fed. Cir. 1996) ("a 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' suffices"); *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (same); *Nelson v. Bowler*, 626 F.2d 853, 857, 206 U.S.P.Q. 881 (C.C.P.A. 1980) (same). In addition, utility need only be shown to be "more likely than not true," not to a statistical certainty. *M.P.E.P.* at § 2107.02, part VII (2004). Considering the evidence as a

whole in light of the relevant standards for establishing utility, Applicants have established at least one specific, substantial, and credible utility for the claimed invention. In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejections under 35 U.S.C. § 112, first paragraph – Enablement

The Examiner also maintains its rejection of pending Claims 1-5 under 35 U.S.C. § 112, first paragraph. Specifically, the Examiner asserts that because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility, one skilled in the art would not know how to use the claimed invention. *Office Action* at 32.

As an initial matter, Applicants submit that in the discussion of the 35 U.S.C. § 101 rejection above, Applicants have established a substantial, specific, and credible utility for the claimed antibodies. The Examiner's rejection is based on lack of utility, which Applicants have fully addressed above. For the reasons set forth in the section addressing the rejection under 35 U.S.C. § 101, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of Claims 1-5 under 35 U.S.C. § 112, first paragraph.

Rejection under 35 U.S.C. §102(b) – Anticipation

The Examiner maintains the rejection of Claims 1-5 as allegedly being anticipated under 35 U.S.C. § 102(b) by Eaton *et al.* (WO 01/16318) (hereinafter Eaton), to which Applicants' claim priority. According to the Examiner, the claimed invention does not fulfill the requirements of 35 U.S.C. § 112, and thus Applicants are not entitled to the benefit of WO 01/16318, and cites the same against Applicants' present application. Applicants respectfully traverse.

Under 35 U.S.C. § 120, an applicant is entitled to the benefit of the filing date of an earlier filed application that discloses the same invention in the manner provided by 35 U.S.C. § 112, first paragraph, provided the applicant properly claims priority to the earlier application. In a preliminary amendment filed on September 3, 2002, Applicants made specific reference to WO 01/16318, claiming priority thereto. WO 01/16318 contains the same disclosure relating to PRO300 and its utilities as the instant application, including the data in Example 18. For the

same reasons detailed above in the Remarks addressed to the rejections under 35 U.S.C. § 101 and 112, Applicants submit that WO 01/16318 is enabling for and adequately describes the claimed invention. Therefore, because Applicants have properly claimed priority to WO 01/16318, and because WO 01/16318 satisfies the requirements of 35 U.S.C. § 112, Applicants are fully entitled to the benefit of the filing date of WO 01/16318 and it cannot be prior art to the present application. Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 102(b).

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: June 13, 2006

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